Italian Botanist 20: 23–40 (2025)
doi: 10.3897/italianbotanist.20.138138
https://italianbotanist.pensoft.net

RESEARCH ARTICLE

Italian Botanist
Published by Società Botanica Italiana

Detection of compounds in the essential oil and extract of *Lycium shawii*: Investigation on their anticancer, antimicrobial, antibiofilm and anti-efflux activities

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Academic editor: Lorenzo Peruzzi | Received 29 September 2024 | Accepted 17 March 2025 | Published 22 August 2025

Citation: Torabi F, Hashemabadi D, Kaviani B, Asadpour L (2025) Detection of compounds in the essential oil and extract of *Lycium shawii*: Investigation on their anticancer, antimicrobial, antibiofilm and anti-efflux activities. Italian Botanist 20: 23–40. https://doi.org/10.3897/italianbotanist.20.138138

Abstract

The tendency has been increasing toward the use of plant secondary metabolites to limit the side-effects of antibiotics and bacterial drug resistance. The present research aimed to investigate the essential oil of *Lycium shawii* and to explore its antimicrobial effect. The essential oil was extracted in ethanol and its antimicrobial activity was investigated by the disk diffusion method in order to determine the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). The anti-biofilm, and anti-efflux effects of the essential oil were investigated by the microplate method, and by determining the MIC of ethidium bromide, respectively. The expression level of biofilm and efflux-associated genes, including *bap*, *abeM*, and *adeB* in *Acinetobacter baumannii* treated or not with the essential oil was evaluated by the real-time PCR method. The inhibitory effect of the essential oil on the propagation and viability of MCF-9 cells was studied by the 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. The diameter of the zone of inhibition varied between 14–44 mm and the MIC values against tested bacteria ranged between 3.12–25 mg mL^{-1}. The expression levels of *bap*, *abeM*, and *adeB* were significantly reduced in treated vs untreated bacteria. The IC50 was 4.66 mg L^{-1} for the MCF-7 breast cancer cell line. The essential oil of *L. shawii* has antimicrobial activity and influences the clinical isolates of the bacteria, as well as exerting an inhibitory effect on the MCF-7 cell line.

Keywords

Biological activity, gene expression, medicinal plants, plant secondary metabolites

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Introduction

In addition to antimicrobial resistance, cancer is a chief cause of death across the world (Mirzaie et al. 2017). Most chemical medications are imitations of herbal medications that have synthetically been produced in pharmaceutical laboratories. Genetic changes and the increased number of mutations in the biofilm phase of the bacteria are other reasons for inducing their resistance to antimicrobial or fatal agents (Costerton et al. 1999; Ma and Bryers 2013). The first and most important efflux pump that has been identified in *Acinetobacter baumannii* is *adeABC* which expels antimicrobial or antibiotic agents from the cell by the driving force of protons and protects the bacterial cells from their damage. The efflux pump *adeABC* is composed of three components: outer membrane proteins encoded by *adeA*, membrane fusion proteins encoded by *adeB*, and inner membrane proteins encoded by *adeC* (Coyne et al. 2010, 2011). Many medicinal plants are traditionally used to cure various bacterial infections and cancers (Mohammad et al. 2022). The main goal in preventing cancers by naturally occurring or chemical compounds is to slow down and/or suppress the carcinogenic process. This mainly derives from intracellular pathways that cause the abnormal functioning of the cell (Aggarwal et al. 2007).

Lycium sp. pl., from the family Solanaceae, comprises ca. 90 species of thorny shrubs distributed throughout the tropical regions of the world. The Arabian boxthorn, *Lycium shawii* Roem. & Schult. is a medicinal plant with healing properties that is used in Iranian traditional medicine. This species adapted to desert environments and can be found throughout the Arabian Peninsula, and some places in Africa (Kamil 2023). Recent phytochemical studies have shown the presence of various compounds, e.g., terpenoids, alkaloids, and flavonoids, with various biological activities in *Lycium* species. *L. shawii* Roem. & Schult. provided fourteen compounds, including lyciumate, dehydrocostus lactone, costunolide, catechin, lyciumaside, emodin, emodin-8-*O*-β-D-glucoside, aloe-emodin, aloe-emodin-8-*O*-β-D-glucoside, aloe emodin-11-Orhamnoside, chrysophanol-8-*O*-β-D-glucoside, nonacosane-10-ol,betulinic acid, and β-sitosterol glucopyranoside. The compounds may be classified as three sesquiterpene lactones (two phenolic compounds, six anthraquinones, one long chain alcohol), one lupane-type triterpenoid and a steroid (Kamil 2023). Jassim Jabur and Noory Fajer (2023) reported that *L. shawii* contains many nutrients, such as polysaccharides, phenolic acid, carotene, betaine, and flavonoids, which possess many advantages, such as antioxidant, anti-radiation, anti-cancer, anti-aging properties, promoting hematopoiesis, brightening eyes, etc. Many studies have confirmed that food additives rich in antioxidants play an important role (Pollini et al. 2019). In the prevention and treatment of many diseases, as the fruits of the *L. shawii* plant contain sugars, vitamins C and E and carotenoids, flavonoids, and betain, which are effective antioxidants that work to curb free radicals. It increases the activity of enzymatic antioxidants, and thus reduces the oxygenation of cells that cause apoptosis (Jassim Jabur and Noory Fajer 2023). However, no investigation has ever been reported on the use of the leaf extract of *L. shawii* as an alternative source of naturally occurring antioxidant and antimicrobial agents to combat multi-drug/pan-drug resistant (MDR/PDR) pathogens (Ali et al. 2020). So, the present research aimed to detect the compounds in the essential oil and extract of this plant species in order to explore their anticancer, antimicrobial, anti-biofilm, and anti-efflux activities.

Materials and methods

Plant material

The Arabian boxthorn (*Lycium shawii*) plants were collected from Minab County in Hormozgan province, Iran in the autumn of 2019. Identification of this species was carried out by Isfahan Center for the Research of Animal Species and Natural Resources Herbarium with IDs No. 9695 and 10491. The leaves and stems were cut from the mother plants and dried.

Preparation of the plant extract

For extraction, 10 g of dried leaf and stem powder were mixed with 100 mL of 70% ethanol and mixed gently for 24 h in a dark room (at 21 ± 2 °C) with a shaker. The resulting extract was filtered with Whatman No. 2 filter paper and then in order to evaporate the solvent, the extract was transferred to a distillation apparatus for 2 h. In order to remove possible bacteria , the extract was sterilized by exposure to UV light for 20 min. Then the extract was transferred to sealed black containers and kept in the refrigerator (4 °C) until use.

Extraction of essential oil

In order to extract the essential oil from the leaves and stems, 100 g of dry powder was extracted with water by distillation using a Clevenger for 2 h.

Gas chromatography-mass spectrometry (GC/MS) analysis of the extracts and essential oils

After preparing the extract and essential oil from the leaves and stems, the analysis and identification of their active ingredients was done by GC-MS apparatus (Agilent 6890). The column was 60 mm long, 0.25 mm inner diameter and 0.25 μM thickness, (Varian, VF-1 ms type). The temperature program was as follows: initial temperature 50 °C, final temperature 280 °C and temperature gradient 7 °C. The temperature of the injection site was 280 °C and the injection volume was 1 μL. The carrier gas used was 99.99% helium with a flow rate of 1 mL min^{-1}. The MS used was an Agilent 5973 Network, Mass Selective Detector model with an ionization voltage of 70 eV. The ionization method was Electron Impact (EI) and the temperature of the ionization source was 230 °C.The spectra were identified with the help of index and inhibition time and the comparison of this element with the mass spectra of standard compounds and the information available in NIST Ms Spectral Search Program and Wiley Mass Spectral Library.

Antimicrobial activity assays

The antimicrobial effect of the leaf and stem extracts and essential oil of *L. shawii* was examined against the standard strains of *Escherichia coli* PTCC 1789, *Pseudomonas aeruginosa* PTCC 1791, *Staphylococcus aureus* PTCC 1826, and *Acinetobacter baumannii* PTCC 1919.

Disk diffusion method

For this assay, standard paper (Whatman, #1) disks with a diameter of 6 mm were impregnated with 25 µL (10 mg per disc) of extracts and essential oil. Then, the disk diffusion method was used to check their antimicrobial effects on the above-mentioned strains by measuring the zone of inhibition.

MIC and MBC methods

MIC was determined by the broth macro-dilution method. Different concentrations of the extracts and essential oil (0.78–50 mg mL^{-1}) were added to fresh standard culture (1.5×10^6 CFU ml^{-1}) of *P. aeruginosa*, *S. aureus*, and *E. coli* and incubated at 37 °C for 24 h. To determine the MBC, the tubes that were found to be clear at the MIC stage were cultured on an agar medium to detect the minimum concentration that would hinder the formation of the colony.

Assay of anti-biofilm activity

The test isolates were first incubated in tryptic soy broth containing 1% glucose at 37 °C for 20 h, and then, a suspension of 1.5×10^8 CFU ml^{-1} of test bacteria was prepared. In each well of a 96-well microplate, 100 µL of Muller-Hintone broth medium containing different concentrations (1.56–25 mg mL^{-1}) of essential oil was poured and 20 µL of prepared bacterial suspension were added to each well. Essential oil was not added to one well, which was considered as a control. The microplates were incubated at 37 °C for 24 h after which the wells were washed with PBS three times to separate the planktonic bacteria and subsequently fixed by methanol for 20 min. Then, 200 µL of 0.02% crystal violet was added to the wells and after 5 min, the color was washed with distilled water. After drying the plates, the biofilm was quantitatively analyzed by adding 200 µL of 33% glacial acetic acid to each well and reading its optical density (OD) at 492 nm with an ELISA reader (Iranpoor et al. 2019).

Assay of the anti-efflux effect by the Cart-Wheel method

Acinetobacter baumannii was cultured in Mueller Hinton broth medium containing 0.0004 g L^{-1} of ethidium bromide (EtBr) and 1/2MIC- 1/32MIC concentrations of *Lycium* leaf essential oil. The prepared suspension was incubated at 37 °C for 4 h; it was then cultured on a Muller-Hinton agar medium, and the plates were incubated at 37 °C for 24 h. The cultures containing carbonyl cyanide m-chlorophenyl hydrazone (CCCP) and 0.0004 g L^{-1} EtBr were used as control. CCCP disrupts the proton gradient across the inner mitochondrial membrane, preventing ATP production and leading to increased oxygen consumption and heat generation. This substance is

utilized in biochemical and microbiological research to study the mechanisms of energy production in cells. The amount of fluorescence caused by the presence of EtBr was checked with a UV transilluminator. The fluorescence intensity on the bacterial culture was regarded as the amount of inhibition of EtBr pumping. The active efflux pump is present in culture lines that do not have fluorescent properties due to EtBr pumping (Hammer et al. 1999).

Effect on cell proliferation and survival by the 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method

First, the MCF-7 cell line was cultured in a 96-well plate (500 cells / 100 µL) and incubated in a CO_2-containing incubator at 37 °C for 24 h. Then, leaf essential oil was dissolved in the culture medium at a concentration of 300 mg mL^{-1}. The essential oil was added to the cell-containing wells at a concentration of 1.56–50 mg mL^{-1}. Following treatment, the plates were incubated for 24–72 h. Subsequently, 10 µL of MTT dye were added to each well, and the plates were incubated for 4 h. Purple formazan crystals were then dissolved in dimethyl sulfoxide (DMSO), and the OD was measured at 540–630 nm, which is directly proportional to the number of living cells.

Gene expression analysis

For this purpose, the presence of *BAP*, *abeM*, *and adeB* genes in *Acinetobacter baumannii* isolates was first confirmed by the polymerase chain reaction (PCR) method. Real-time qPCR was was used to evaluate the expression level of the genes in the isolates of *A. baumannii* treated with a sub-MIC (1.56 mg mL^{-1}) concentration of *L. shawii* leaf essential oil. Total RNA extraction was done using Kiagene RNA extraction kit. cDNA was synthesized from the extracted RNA using random hexamer primers through the steps recommended by the manufacturer (Fermentas, USA). The synthesized cDNA was used for reverse transcription reaction. PCR was performed in a volume of 20 mL using the kit of Aus Diagnostics, Australia and according to the manufacturer's instructions. To perform the reaction, 1 µL of cDNA was added to 10 µL of CYBER Green Master Mix (ABI, USA), 0.5 µL of each primer with a concentration of 10 pM and 8 µL of DEPC water. In this study, 16S rRNA gene was used as a standard gene. The studied genes and the nucleotide sequence of the primers used in the real-time PCR are reported in Table 1. Gene expression change was calculated using the $2^{-\Delta\Delta CT}$. This analysis was repeated three times.

Statistical analysis

Data were subjected to statistical analysis by one-way analysis of variance followed by Tukey's test to check the significance at the $P \leq 0.05$ level.

Results

Compounds detected in the leaf and stem essential oils and extracts

The compounds detected by GC-MS in the leaf and stem essential oils are reported in Table 2. Nine compounds were identified in the leaf and eight in the stem. The most abundant compounds in the leaf essential oil were hexadecanoic acid (8.08%), neophytadiene (7.54%), and phytol (6.11%). The most abundant compounds in the stem essential oil were 2-methoxyphenol (7.01%), and 3-(2-naphthyl)-1-butene (5.15%). The GC-MS chromatograms are shown in Figs 1, 2. The compounds identified in the leaf and stem extracts are presented in Table 3. Among the compounds identified in the leaf extract, the most abundant ones were 15-octadecatrien-1 (12.29%), phytol (10.39%), and 9,12,15-octadecatrienoic acid (9.84%). Nine compounds were identified in the stem extract. The main compounds included toluene (17.41%), hexadecanoic acid (12.06%), and cyclotrisiloxane (10.50%).

Table 1. Studied genes and nucleotide sequence of the primers used in the real-time PCR reaction.

Gene name	Primer	Sequence (5'®3')
BAP	F	TAGACGCAATGGATAACG
	R	TTAGAACCGATAACGATACC
abeM	F	TGCCAATTGGTTTAGCTGTG
	R	TACTTGGTGTGCGGCAATAA
adeB	F	GGATTATGGCGACTGAAGGA
	R	AATACTGCCGCCAATACCAG

Table 2. Chemical compounds detected in the leaf and stem essential oils of *L. shawii*.

Essential oil	Compound	Abundance (%)	Time of detection (min)
Leaf	Phenol	3.52	7.04
	Phenol, 2,4-dimethyl	3.63	9.70
	Indole	4.10	11.72
	7-Methylindole	3.19	13
	Neophytadiene	7.54	18.41
	Hexadecanoic acid	8.08	19.54
	Phytol	6.11	20.97
	9-Pentadecadien-1	5.27	21.15
	Eicosane	5.52	30.08
Stem	Phenol,2-methoxy	7.01	8.69
	2-Methoxy-4-methylphenol	4.09	10.33
	4-vinyl-2-methoxy-phenol	5.05	12.05
	2H-Pyran-2,4(3H)-dione	3.26	13.73
	3-(2-naphthyl)-1-butene	5.15	14.72
	Hexadecanoic acid	5.10	19.52
	Isomaturnin	3.02	21.56
	Heptacosane	4.33	25.68

Table 3. Chemical compounds detected in the leaf and stem extracts of *L. shawii*.

Extract	Compound	Abundance (%)	Time of detection (min)
Leaf	Neophytadiene	4.95	18.42
	Hexadecanoic acid	6.19	19.52
	Phytol	10.39	20.97
	9,12,15-Octadecatrien-1	12.29	21.13
	9,12,15-Octadecatrienoic acid	9.84	21.37
	1,2-Benzenedicarboxylic acid	6.97	24.38
	Heptacosane	5.03	25.68
	Eicosane	4.32	30.07
	Cyclotrisiloxane	4.58	32.75
	Methyl-2 Phenylindole	6.16	34.66
Stem	Toluene (Benzene, Methyl)	17.41	3.57
	Butyl Acetate	8.70	4.17
	Xylene	9.83	5.18
	4H-1,2,4-Triazol-4-Amine	5.83	10.75
	9-Nonamethylpent	5.65	14.74
	Hexadecanoic Acid	12.06	19.50
	2-Chloroethyl Linoleate	7.72	21.32
	Di-(2-Ethylhexyl)Phthalate	10.16	24.38
	Cyclotrisiloxane	10.50	25.68

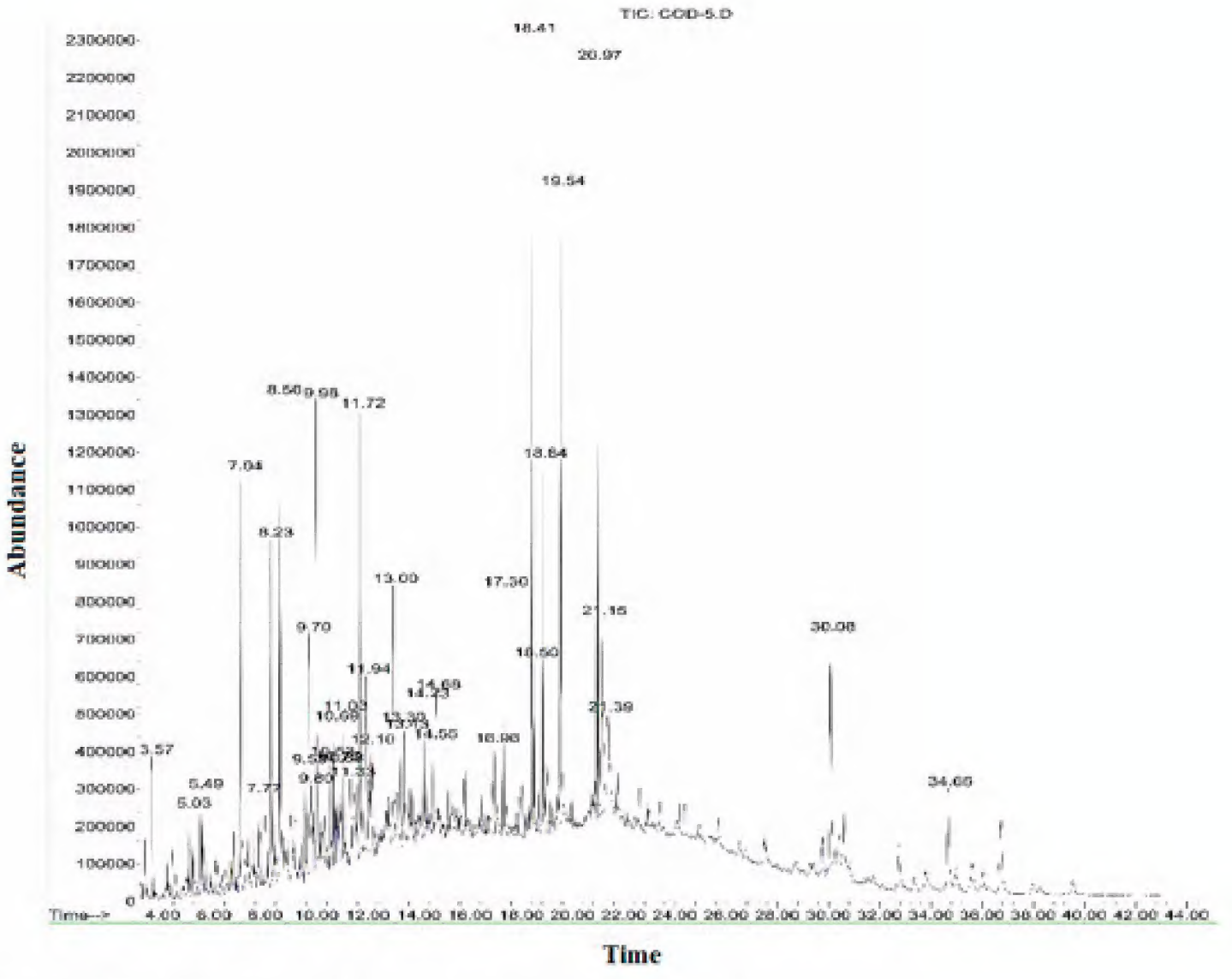

Figure 1. GC-MS chromatogram of *L. shawii* leaf essential oil.

Antimicrobial activity

Table 4 presents the results of the anti-microbial assay of the leaf and stem extracts and essential oils of *L. shawii* by the disk diffusion method. The lowest zone of inhibition caused by the extracts and essential oils was related to *S. aureus*, reflecting its higher resistance. The leaf extract demonstrated the highest efficacy in inhibiting the examined bacteria compared to leaf and stem essential oils, as well as the stem extract (Table 4).

The results of MIC revealed the lack of a significant difference between the extracts and essential oils in suppressing the growth of *E. coli* (MIC = 6.25 mg mL^{-1}) (Table 5). The most effective compound in suppressing the growth of *P. aeruginosa* was the stem extract (MIC = 6.25 mg mL^{-1}). The leaf essential oil (MIC = 3.12 mg mL^{-1}) and the leaf and stem extracts (MIC = 25 mg mL^{-1}) were the most and least effective in inhibiting the growth of *A. baumannii*, respectively. The leaf essential oil and leaf and stem extracts (MIC = 3.12 mg mL^{-1}) were more effective in inhibiting the growth of *S. aureus* than the stem essential oil (MIC = 6.25 mg mL^{-1})

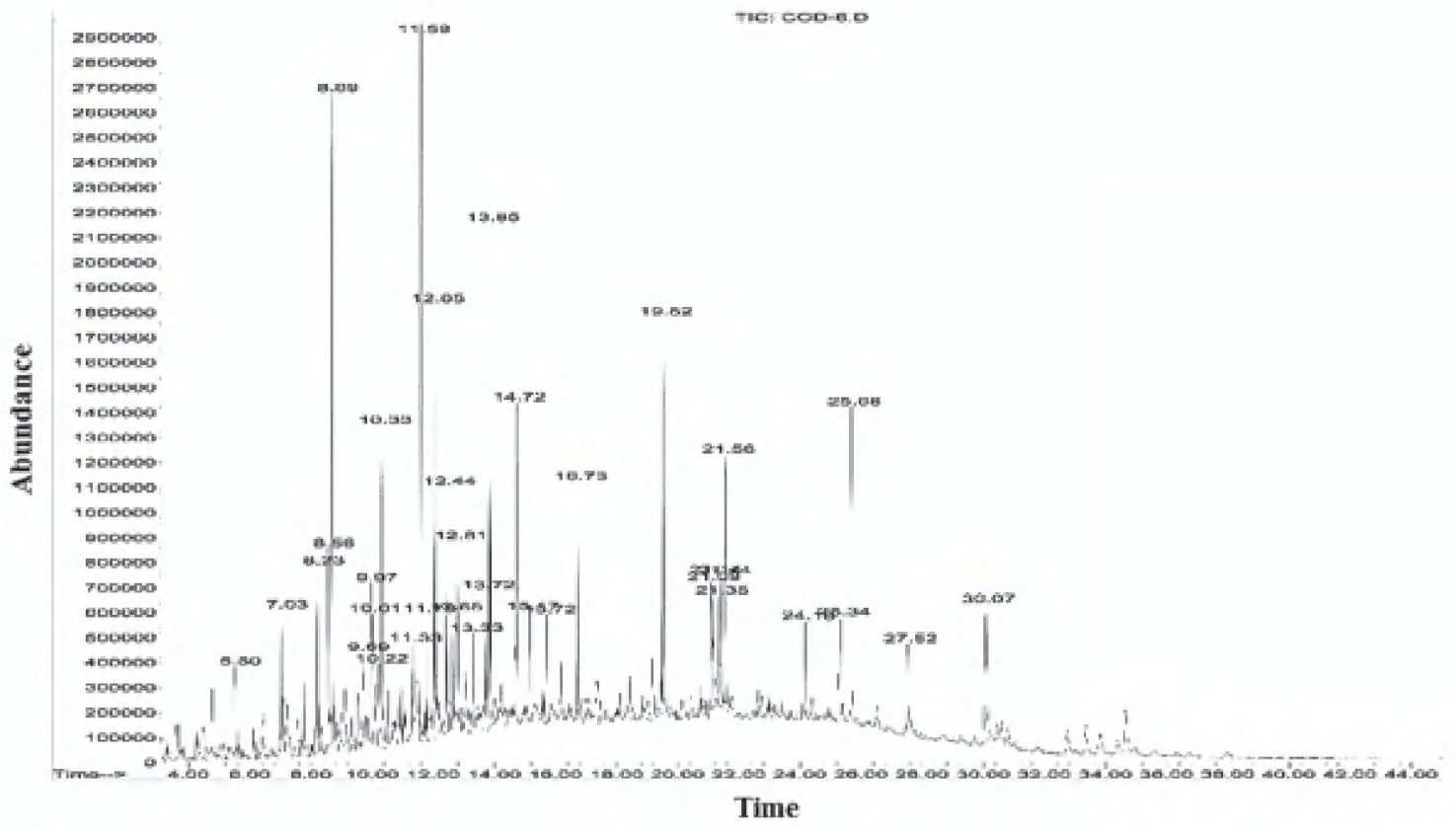

Figure 2. GC-MS chromatogram of*L. shawii* stem essential oil.

Table 4. Antimicrobial effect of the leaf and stem essential oils and extracts of *L. shawii* on *E. coli*, *P. aeruginosa*, *A. baumannii*, and *S. aureus* with the disk diffusion method.

	Stem essential oil	Leaf essential oil	Stem extract	Leaf extract
	Non-growth halo diameter (mm)			
E. coli	25	36	29	44
P. aeruginosa	21	41	30	44
A. baumannii	20	38	35	41.5
S. aureus	14	17	15	18.5

Table 5. MIC and MBC of the leaf and stem essential oils and extracts of *L. shawii* against *E. coli*, *P. aeruginosa*, *S. aureus*, and *A. baumannii*.

	E. coli		*P. aeruginosa*		*A. baumannii*		*S. aureus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
	(mg ml^{-1})							
Leaf essential oil	6.25	12.50	25	50	3.12	6.25	3.12	6.25
Stem essential oil	6.25	12.50	25	50	6.25	12.50	6.25	12.50
Leaf extract	6.25	12.50	25	12.50	25	50	3.12	6.25
Stem extract	6.25	12.50	6.25	12.50	25	50	3.12	6.25

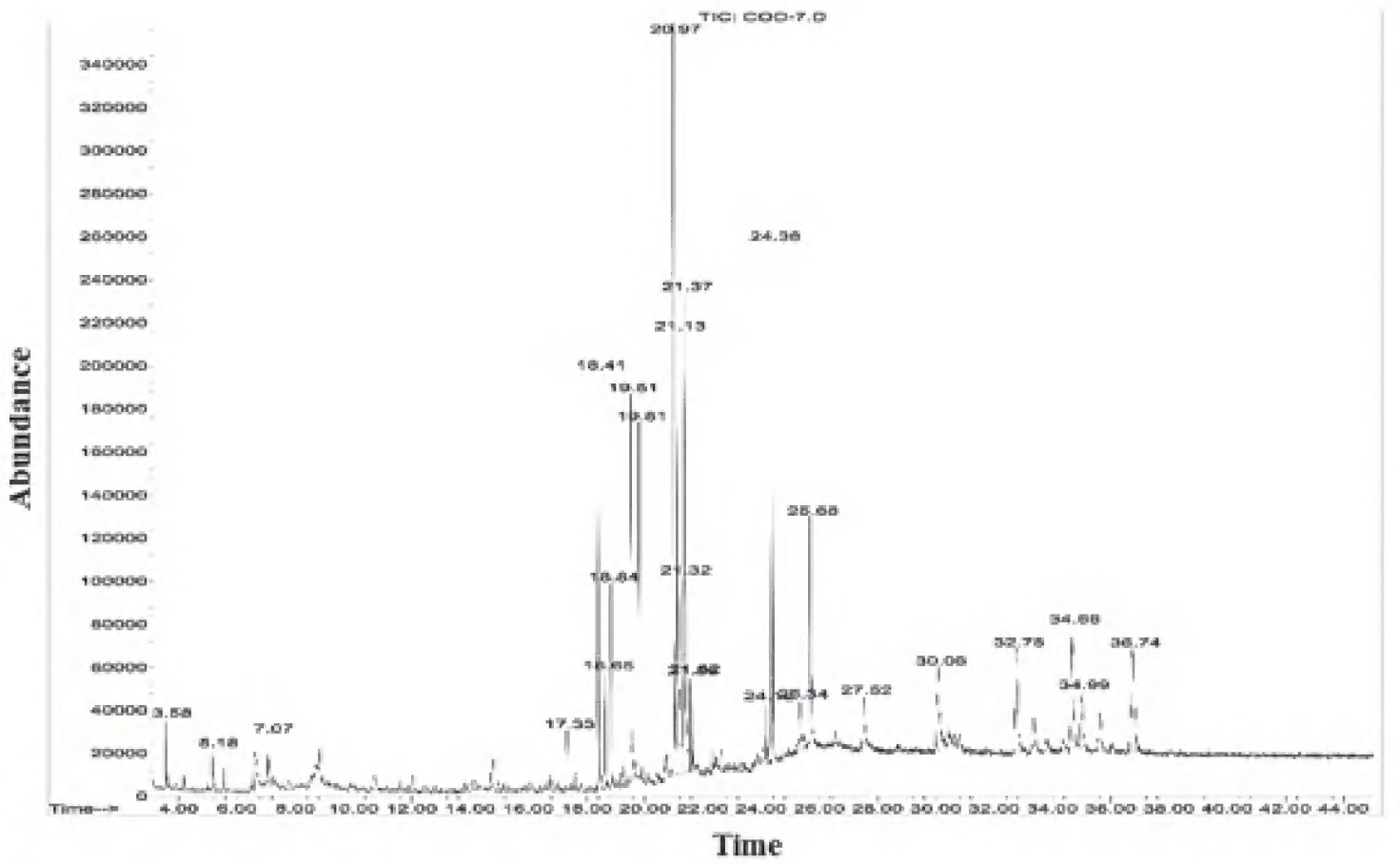

Figure 3. GC-MS chromatogram of *L. shawii* leaf extract.

(Table 5). The results for MBC (Table 5) showed that it was 12.5 mg mL^{-1} for the leaf and stem extracts and essential oils against *E. coli*, which was similar to that for *P. aeruginosa*. Also, *P. aeruginosa* and *A. baumannii* (MBC = 50 mg mL^{-1}) exhibited the highest resistance to the bactericidal effect of the *L. shawii* essential oils and extracts, respectively. The leaf essential oil and extract and the stem extract (MBC = 6.25 mg mL^{-1}) had a stronger bactericidal effect on *S. aureus* than the stem essential oil (MBC = 12.5 mg mL^{-1}) (Table 5, Figs 5, 6).

Effect of leaf essential oil on the inhibition of biofilm formation by *A. baumannii*

The essential oil derived from the leaves demonstrated great efficacy in preventing the development of biofilm by *A. baumannii* at elevated concentrations (Fig. 7).

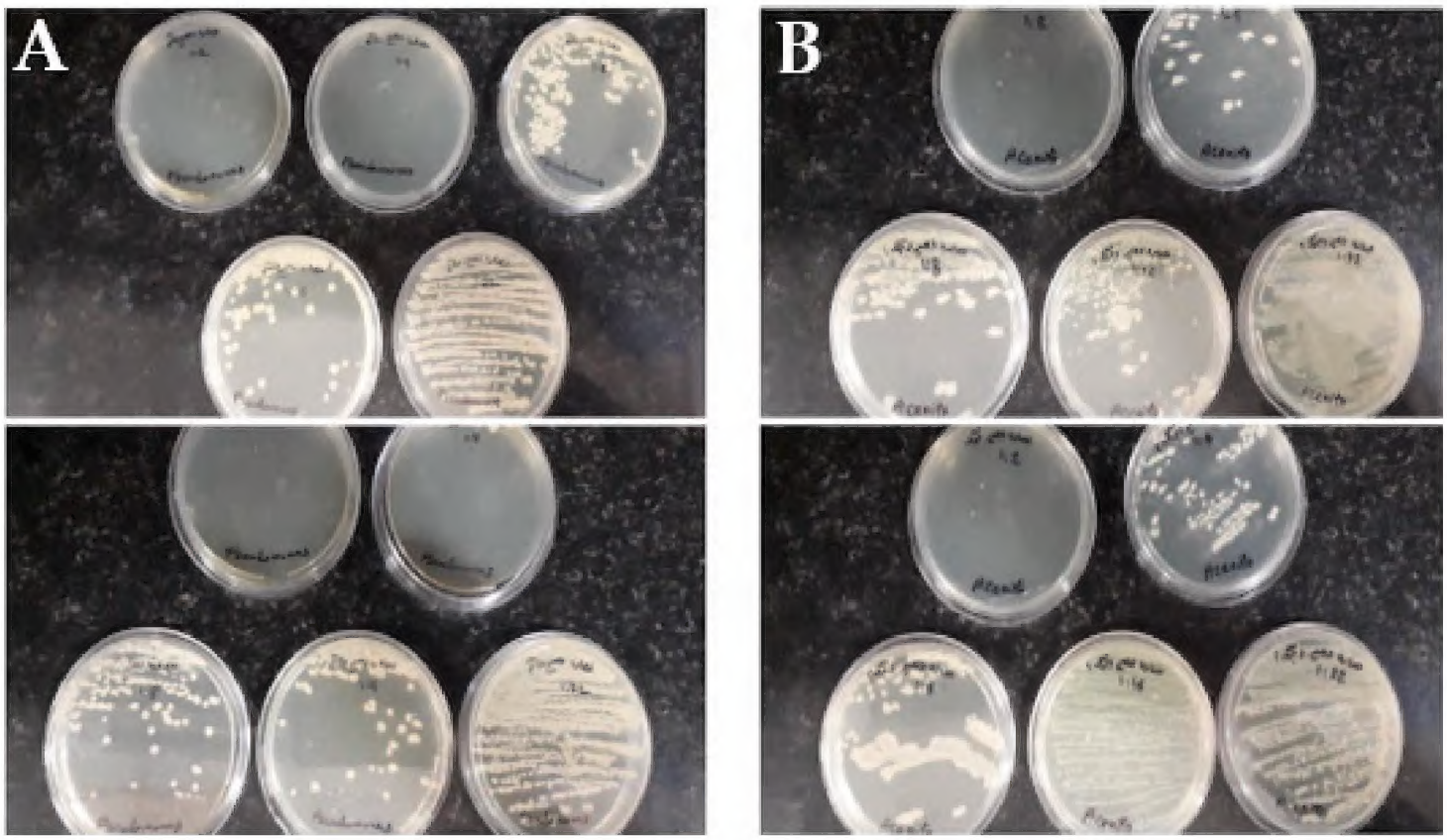

Figure 4. GC-MS chromatogram of *L. shawii* stem extract.

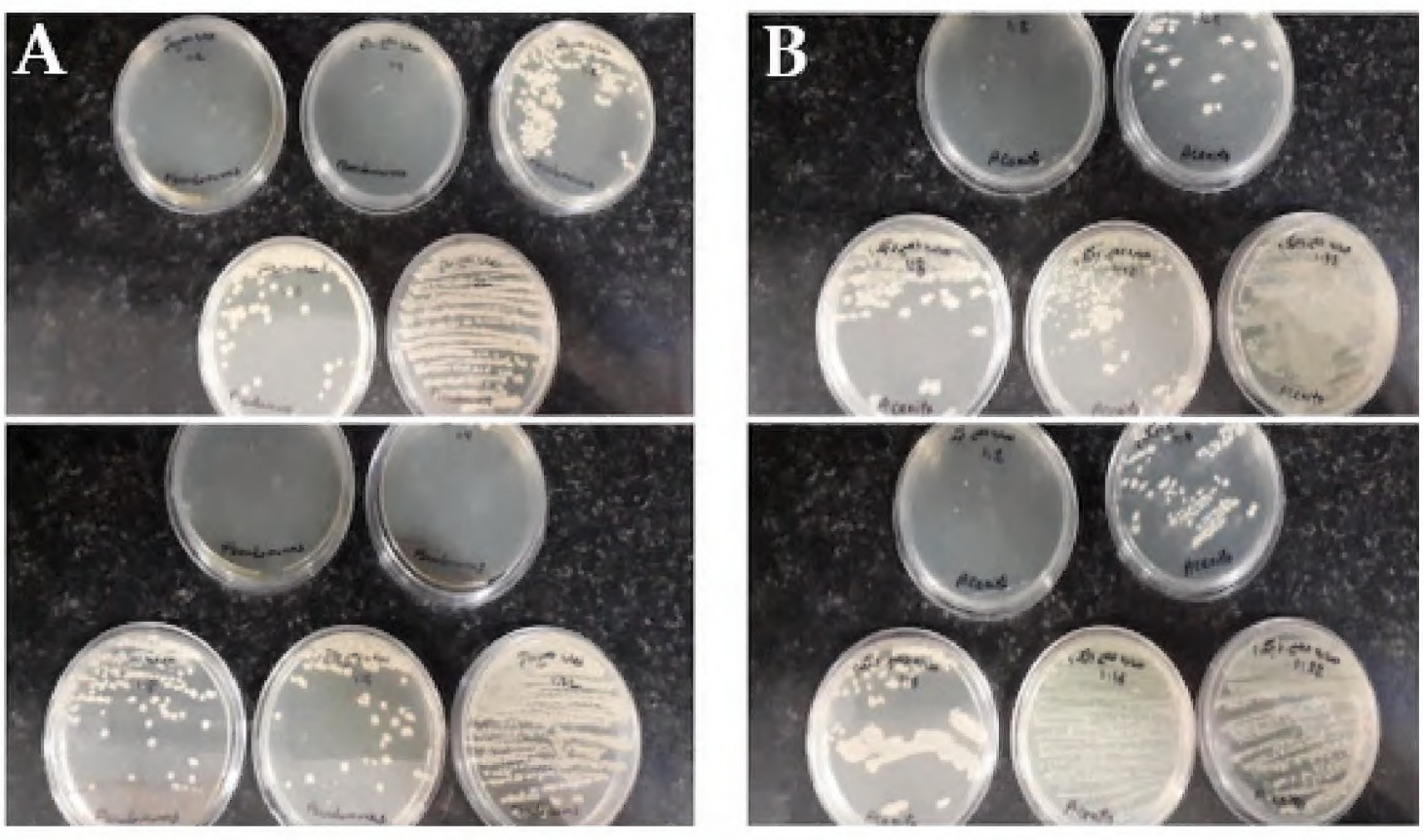

Figure 5. MBC of the *L. shawii* stem extract against *P. aeruginosa* (**A**) and MBC of the *L. shawii* leaf extract against *A. baumannii* (**B**).

Anti-efflux pump effect of the leaf essential oil

According to the results, the sub-MIC concentrations of the essential oil reduced the rate of EtBr pumping by the bacteria. (Fig. 8). Furthermore, treatment of *A. baumannii* with sub-MIC concentration of the leaf essential oil caused a 25%, 70%, and

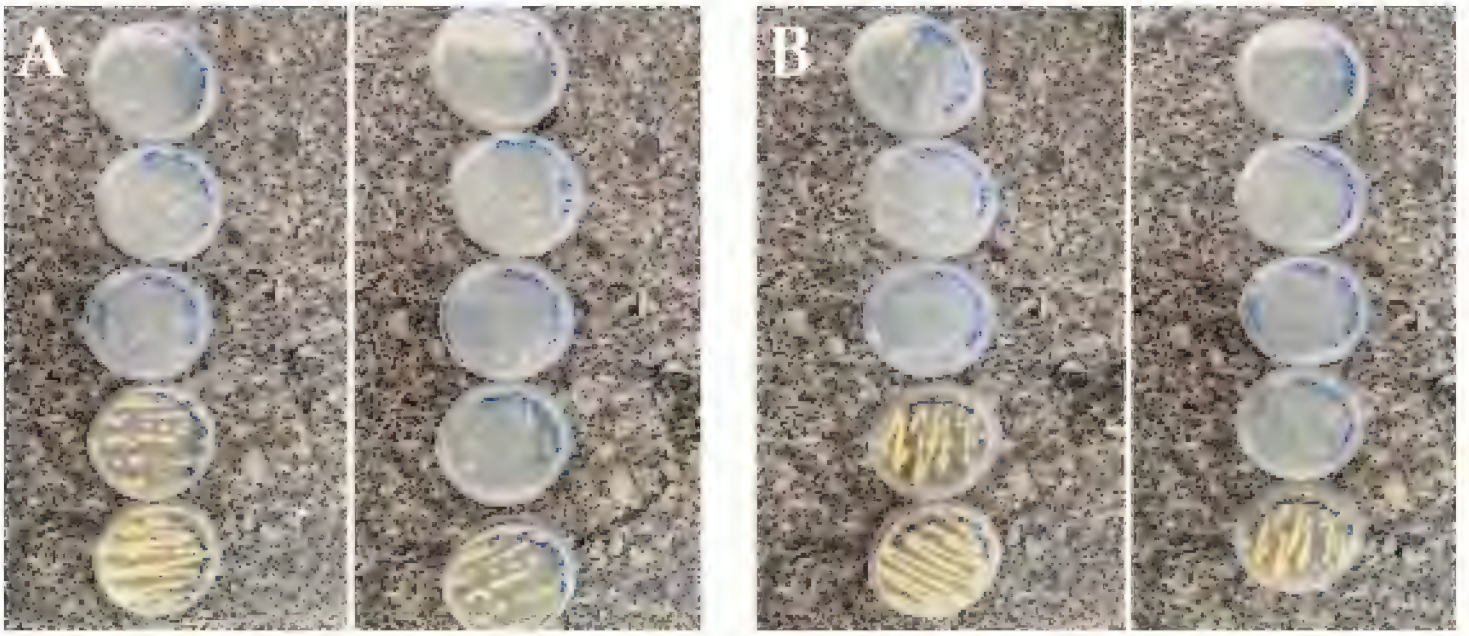

Figure 6. MBC of the *L. shawii* leaf extract against *E. coli* and *S. aureus* (**A**) and MBC of the *L. shawii* stem extract against *E. coli* and *S. aureus* (**B**).

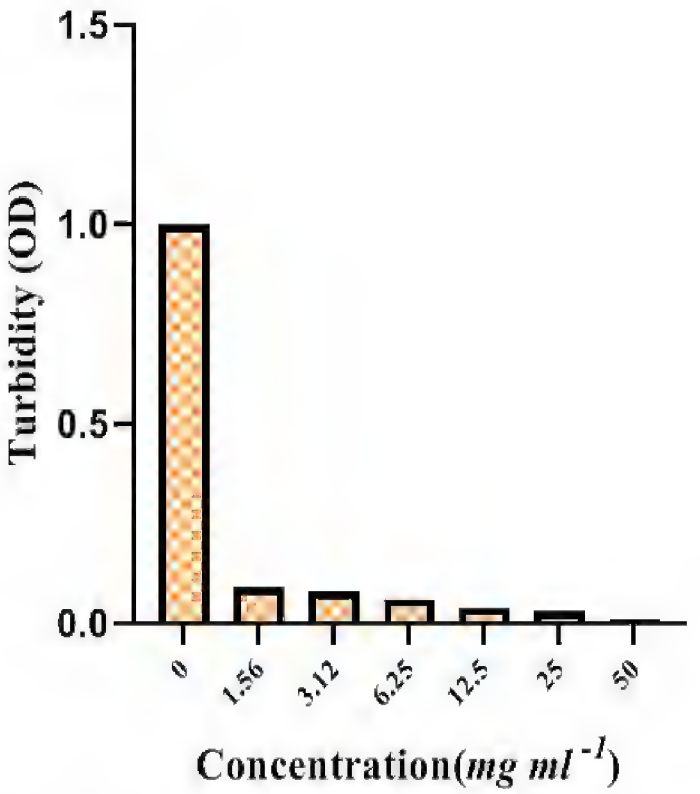

Figure 7. Effect of *L. shawii* leaf essential oil on biofilm formation by *A. baumannii.*

65% reduction in the expression levels of *bap*, *abeM*, and *adeB*, respectively compared to the untreated control (Fig. 9). These genes are involved in bacterial efflux and biofilm formation.

Effect on the MCF-7 breast cancer cell line

The effect of the leaf essential oil on the MCF-7 breast cancer cell line revealed that the toxicity of the essential oil for this line was dose-dependent, so higher doses reduced its viability and increased death. The IC50 value was 4.66 μg/mL^{-1} for 48 h (Fig. 10).

Figure 8. Fluorescent image of an agar plate streaked with *A. baumannii* and containing 0.0004 g L^{-1} ethidium bromide and different sub-MIC concentrations of *L. shawii* leaf essential oil.

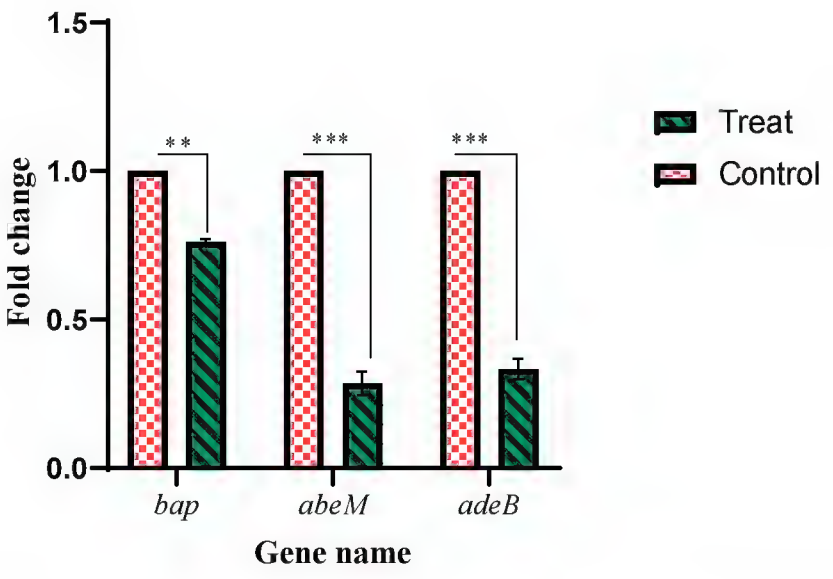

Figure 9. The expression of *adeB*, *abeM*, and *bap* in *A. baumannii* treated with the *L. shawii* leaf essential oil.

Discussion

The research detected the compounds in the essential oils and extracts of the leaf and stem of *L. shawii* and studied their antimicrobial activity. In this regard, it is important to exploit the potential of indigenous and available plant species. *L. shawii* is native to southern regions of Iran and is regarded as a major nutritional and medicinal source. This medicinal plant is used to treat a wide range of diseases and its extract has been reported to have antimicrobial and antioxidant effects (Yao et al. 2018; Ur Rehman et al. 2020).

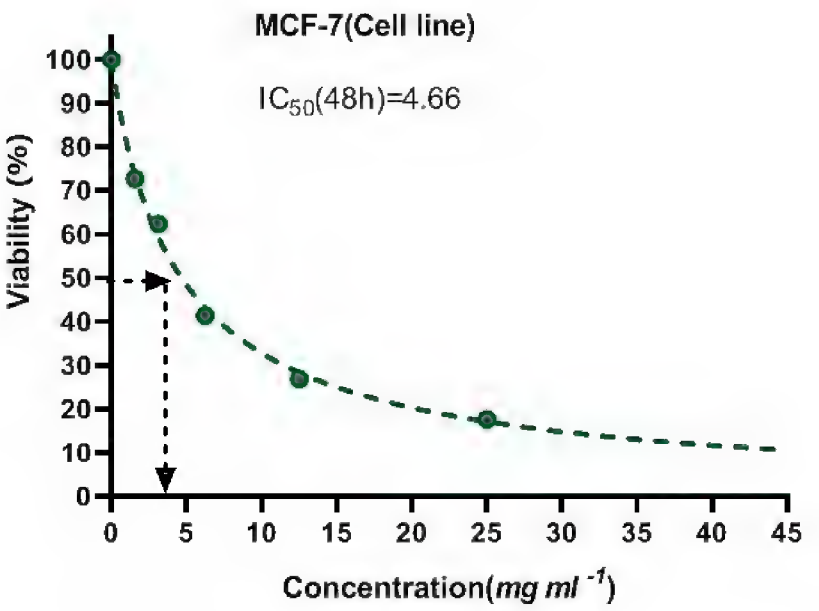

Figure 10. The effect of the *L. shawii* leaf essential oil the viability of MCF-7 breast cancer cell line.

Eight to 10 compounds were detected in the leaf and stem essential oil extracts. Ali et al. (2020) identified 10 compounds in the methanolic leaf extract of *L. shawii* by GC/MS. Coumaric acid, apigenin, and fisetin were the three main compounds. Mocan et al. (2014) reported that chlorogenic acid, rutin, and *p*-coumaric acid were the most abundant compounds in two *L. shawii* species. The difference in the quantity and quality of essential oil and extract compounds of a plant species in different investigations can be caused by climatic and ecological conditions (Foroozeh and Mirdeylami 2019). Examination of the zone of non-growth of bacteria showed that the leaf extract and then the leaf essence had the greatest effect in inhibiting the growth of all four investigated bacteria. The GC-MS analysis revealed that 9,12,15-octadecatrien-1, phytol and 9,12,15-octadecatrienoic acid were the main constituents of the leaf extract and hexadecanoic acid, neophytadiene and phytol were the main constituents of the leaf essential oil. Phenolic compounds, terpenoids, and fatty acids that make up plant extracts or essential oils play a decisive role in their antimicrobial activity. Phytol, as a diterpenoid, has antibacterial and antioxidant properties. Phytol causes cell death in bacteria by increasing oxidative stress and DNA damage (Lee et al. 2016). Fatty acids have strong antibacterial and antifungal effects (Ouattara et al. 1997). 9,12,15-octadecatrien-1 and 9,12,15-octadecatrienoic acid are unsaturated fatty acids that the presence of three double bonds in their structure provides special antioxidant and biological properties. The antimicrobial effect of decanoic acid (fatty acid) (Shaaban et al. 2021) and neophytadine (diterpenoid and plant aromatic substances) (Gonzalez-Riverl., 2023), which were the main components of essential oil and leaf extract of *L. shawii*, have been reported in previous studies. In general, the unique potentials of the compounds that make up the extracts and essential oils of different plants by using different mechanisms, including damage to the cell membrane, deactivation of enzymes and DNA destruction, cause disruption in the growth and death of bacteria and pathogens (Sethunga et al. 2023). Therefore, the inhibitory effect of *L. shawii* leaf extract and essential oil on the growth of bacteria can be related to the unique characteristics, including the antimicrobial and antioxidant effect of these substances.

The results of the disk diffusion method indicated that *E. coli* and *P. aeruginosa* were the most sensitive bacteria to the leaf extract and *S. aureus* was the least sensitive to the leaf and stem extracts and essential oils. The lack of effect of leaf and stem extracts and essential oils on the growth of *S. aureus* could be due to differences in phytochemical compositionand the effective dose of antimicrobial compounds on the growth of these bacteria,. Ali et al. (2020) reported that phenols and flavonoids were the main factors responsible for the antioxidant and antimicrobial activity of the *L. shawii* leaf extract. These researchers reported that the leaf extract of *L. shawii* suppressed the growth of *S. aureus* and *P. aeruginosa*.

The growth inhibition halo in the disk diffusion test was recorded as < 15 mm for both *L. chinense* and *L. barbarum* against *S. aureus* and *E. coli* (Mocan et al. 2014). It has been reported that gram-negative bacteria are more resistant to the penetration of antibacterial agents (extracts and essential oils of medicinal plants) than Gram-positive bacteria due to the presence of an external membrane structure (Ali et al. 2023). In the present study, among Gram-negative bacteria, the best MIC = 3.12 and MBC = 6.25 were recorded for *A. baumannii* with the use of leaf essential oil. *A. baumannii* is pathogenic for humans and is the main cause of nosocomial infections due to its high resistance to a wide range of antibiotics. The MIC and the MBC of the leaf essential oil, leaf extract and stem extract were the same for the Gram-positive *S. aureus*, and this bacterium was most sensitive to *L. shawii* leaf and stem extract., This indicatesthe high sensitivity of Gram-positive bacteria compared to Gram-negative ones towards antimicrobial compoundsm, considering that it had the lowest aura of non-growth against the tested antimicrobial agents. Determining the sensitivity of this bacterium to the essential oil and extract of *L. shawii* needs further investigation. It is a Gram-negative and multi-drug resistant bacterium, and one of its most important characteristics is the formation of biofilms at different levels. Biofilm formation increases bacterial resistance to antibiotics a thousand times (Roy et al. 2022). Aflexia pump is a defense mechanism in bacteria, whose activity causes the release of toxic substances and antibiotics from the cell and increases the resistance of bacteria against adverse conditions (Temgoua and Wu 2018). Biofilm formation and efflux pump activity in *A. baumannii* are the most important reasons for the resistance of this bacterium against a wide range of antibiotics. Essential oils and extracts play a significant role in inhibiting biofilm formation and reducing antibiotic resistance of bacteria (El Tarabily et al. 2021). In our research, the best MIC was 3.12 for the *L. shawii* leaf essential oil against *A. baumannii* that is pathogenic to humans. It is a main cause of hospital infections due to its high resistance to a wide range of antibiotics. Present results also show that the leaf essential oil of *L. shawii* reduced the expression of the gene involved in biofilm formation (*bap*) and the genes encoding the efflux pump (*abeM* and *adeB*).

The increased expression of efflux pump genes plays an essential role in the resistance of *A. baumannii* strains (Pidock 2006; Ardestani et al. 2021). It was revealed by Nemec et al. (2007) that the bacterial strains in which the expression of *adeM* and *adeABC* was at higher levels exhibited higher resistance to antimicrobial factors. In the present study, the leaf essential oil of *L. shawii* significantly inhibited the expression of

abeM, *bap*, and *adeB* in *A. baumannii*, which can be a reason for the high susceptibility of this bacterium to the leaf and stem essential oils of *L. shawii*.

It was found that the application of the leaf essential oil reduced the growth of the MCF-7 breast cancer cell line. Similarly, Gaweesh et al. (2015) reported that the application of the *L. shawii* methanol extract suppressed the growth of breast cancer cells. Du et al. (2022) stated that *Lycium barbarum* L. contains polysaccharides that have anticancer properties. Hexadecanoic acid is the main compound identified in the essential oil of *L. shawii* leaves, which has been reported to have anti-cancer properties in previous studies (Budi et al. 2022). According to the results of this research, the essential oil of *L. shawii* leaves can be introduced as an antimicrobial, and anticancer agent.

Conclusion

Based on our results, it can be stated that the plant extract and essential oil of *L. shawii* have antimicrobial activity on several clinical isolates of bacteria. It can be inferred that they can be used alone or in combination with antibiotics to treat infections caused by resistant bacteria and that the cytotoxicity of the leaf essential oil of this plant species can influence the growth of cancer cell lines. These results warrant the need for further studies.

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